REMARKS

Amendments to the Claims

Claims 1, 3-22, 45-47, 56 and 57 are pending. The Applicants respectfully ask the Examiner to replace all prior versions and listings of claims in the present application with the listing of claims currently provided. Claim 45 was amended. The Applicants hereby state that all amendments do not add new subject matter to the specification.

Support for Claim 45 can be found throughout the specification, such as, *e.g.*, pg. 8, ¶ 22-23; pg. 10, ¶ 34; pg. 11, ¶ 36; pg. 14, ¶ 52; pg. 15, ¶ 53; pg. 30, ¶ 120.

Specification Objection

The Examiner has objected to the amendment to the first paragraph alleging that the Applicants have failed to claim a relationship between the present specification and co-pending U.S. patent application 10/757,077. The Applicants respectfully ask for reconsideration under 37 C.F.R. § 1.111.

According to MPEP § 201.11(III)(G) an applicant may cancel a claim to priority by amending the specification to remove the benefit claim.

The Applicants respectfully submit that they are not claiming priority to co-pending U.S. patent application 10/757,077. The Applicants amended the first paragraph to remove the benefit claim, and thus the claim to priority has been canceled. Thus, the Applicants respectfully request withdrawal of the objection to the first paragraph amendment.

Rejections Pursuant to 35 U.S.C. § 112, ¶ 1 Enablement

The Examiner has rejected Claims 45-47 and 57 as allegedly lacking enablement under 35 U.S.C. § 112, ¶ 1. Specifically, the Examiner contends that the specification is not enabling for identifying compounds that increase or decrease a biologically persistence of all

Clostridial toxins by evaluating the intracellular localization of BoNT/A light chain. The Applicants respectfully ask for reconsideration under 37 C.F.R. § 1.111.

Currently amended Claim 45 recites, in part, "a method of identifying a compound that reduces or increases a biological persistence of a BoNT/A." Thus, the Applicants submit that the present specification provides adequate enablement for the presently claimed method and respectfully request withdrawal of the 35 U.S.C. § 112, ¶ 1 enablement rejection against Claims 45-47 and 57.

Rejections Pursuant to 35 U.S.C. § 112, ¶ 1 Written Description

The Examiner has rejected Claims 3, 4, 46 and 47 as allegedly lacking written description under 35 U.S.C. § 112, ¶ 1. The Examiner contends that the limitation "about 20% to about 300% increase" for Claims 3 and 46, and the limitation "about 10% to about 90% reduction" for Claims 4 and 47 do not appear in the present specification or original claims and are thus considered new matter. The Applicants respectfully ask for reconsideration under 37 C.F.R. § 1.111.

According to MPEP § 2163, to satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail that a person of ordinary skill in the art can reasonably conclude that the inventor had possession of the claimed invention. The subject matter of the claim need not be described literally (*i.e.*, using the same terms or *in haec verba*) in order for the disclosure to satisfy the description requirement. Possession of the claimed subject matter can be shown through express, implicit, or inherent disclosure.

First, the Applicants respectfully submit that support cited for the amendments to Claims 3, 4, 46 and 47 is found at pg. 24, ¶ 109; pg. 38, ¶ 156; and pg. 70, ¶ 285 of U.S. Patent Application Serial No. 10/757,077 (Attorney Docket No. 17355 CIP3 (BOT), filed January 14, 2004), a patent application that is incorporated by reference in the present specification, see, ¶ 1. According to *MPEP* § 2163.07(b):

> Instead of repeating some information contained in another document, an application may attempt to incorporate the content of another document or part thereof by reference to the document in the text of the specification. The information incorporated is as much a part of the application as filed as if the text was repeated in the application, and should be treated as part of the text of the application as filed. Replacing the identified material incorporated by reference with the actual text is not new matter.

This support provides sufficient recitation of the range percentages. For example, ¶ 109, ¶ 221, ¶ 225 and ¶ 285 of U.S. Patent Application Serial No. 10/757,077 indicate that a modified neurotoxin can have an increased biological persistence of about 20% to about 300% longer than a neurotoxin that is not modified. Similarly, ¶ 156 indicates that a modified neurotoxin can have a decreased a biological persistence of about 10% to about 90% that of an unmodified BoNT/A light chain.

Second, the Applicants respectfully submit that the present specification teaches that a compound that decreases the characteristic localization pattern of a botulinum toxin decreases biological persistence of that toxin, whereas a compound that increases the characteristic localization pattern of a botulinum toxin increases biological persistence of that toxin. For example, the present specification teaches that the duration of action of botulinum neurotoxins is related to their subcellular localization, with plasma localization resulting in longer duration, see, ¶ 17. The present specification at ¶ 23 states that "[a] change in the localization pattern of the light chain in the cell following contacting the cell with the test compound indicates that the test compound alters, *i.e.*, inhibits or enhances, the biological persistence of the toxin." The present specification further elaborates on the relationship between biological persistence of a BoNT/A light chain and it localization to a membrane in ¶¶ 24-25; and 52-53 where it discloses that compounds that disrupt membrane localization of a toxin light chain result in reduced biological persistence, whereas compounds that enhance membrane localization of the toxin light chain result in increased biological persistence. Lastly, Example 6 discloses methods for identifying compounds that alter the biological persistence of a Clostridial toxin.

Thus, the present specification discloses that 1) the biological persistence of a modified neurotoxin can be decrease by about 10% to about 90% or increased by about 20% to about 300%; 2) this increased or decreased persistence of the modified neurotoxin is associated with a corresponding increase or decrease of the neurotoxin light chain to a plasma membrane; 3) the disclosed screening methods use this altered plasma membrane localization as a read-out of biological persistence. As such, a person of ordinary skill in the art would understand that 1) a method of identifying a compound that increases a biological persistence of a BoNT/A could be observed as an increase of about 20% to about 300% more BoNT/A light chain localized to the plasma membrane as compared to a control; and 2) a method of identifying a compound that decreases a biological persistence of a BoNT/A could be observed as decrease of about 10% to about 90% reduction in plasma membrane localization of the BoNT/A light chain as compared to a control.

Thus, the Applicants respectfully submit that there is adequate written description support for the recited limitations claimed because the present specification conveys with reasonable clarity to those skilled in the art that, as of the filing date sought, that the Applicants were in possession of the invention as now claimed. Therefore, the Applicants respectfully request withdrawal of the 35 U.S.C. § 112, ¶ 1 written description rejection for Claims 3, 4, 46 and 47.

Rejection Pursuant to 35 U.S.C. § 101 Obviousness-Type Double Patenting

The Examiner has provisionally rejected Claims 1, 3-20, 22, 45-47, 56 and 57 as allegedly being unpatentable over Claim 60 of U.S. Patent Application 10/732,703, Shengwen Li and Kei Roger Aoki, *Lipid Rafts and Clostridial Toxins* (Dec. 10, 2003), hereafter the "Li publication" in view of Judit Herreros et al., *Lipid Rafts Act as Specialized Domains for Tetanus Toxin Binding and Internalization into Neurons*, 12 Mol. Biol. Cell 2947-2960 (2001), hereafter the "Herreros reference," under the judicially created doctrine of obviousness-type double patenting under 35 U.S.C. § 101. The Applicants respectfully ask for reconsideration under 37 C.F.R. § 1.111.

According to *MPEP* § 804(II)(B)(1) and (III), an obviousness-type double patenting rejection pursuant to 35 U.S.C. § 101 is "analogous to [a failure to meet] the nonobviousness requirement of 35 U.S.C. 103" and the "analysis employed in an obviousness-type double patenting rejection parallels the guidelines for analysis of a 35 U.S.C. § 103 obviousness determination." Two significant differences are that 1) the patent or co-pending patent application underlying the double patenting rejection is not considered prior art; and 2) a double patenting rejection must rely only on a comparison with the claims in the patent or co-pending patent application. Thus, analysis of the obviousness-type double patenting rejection against Claims 1, 3-20, 22, 45-47, 56 and 57 will be analyzed under 35 U.S.C. § 103 rules.

Li and Herrero combination makes Li unsatisfactory for its intended use.

According to MPEP §2143.01, if proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. The Applicants respectfully submit that it would not be obvious for one of ordinary skill in the art to combine the cited references because combining the Li publication with the Herrero reference would render the internalization assay disclosed in the Li publication as inoperable for its intended use since such a combination would prevent the ability of the assay to determine any effects on toxin internalization.

During the intoxication process, a Clostridial toxin initially binds to a cell surface receptor and the toxin-receptor complex is internalized into the cell by endocytosis, see present application at ¶¶ 13-14. Subsequently, while in the endosome, the heavy chain of the toxin forms a pore and the light chain is released into the cytoplasm, see present application at ¶¶ 13-14. Released of the light chain into the cytoplasm allows the toxin to interact with its target SNARE protein and catalyzes the proteolytic cleavage of the SNARE protein, see present application at ¶¶ 15-16.

The presently claimed methods are directed toward a cell-based light-chain localization assay useful for identifying a compound that either reduces or increases a biological

persistence of a BoNT/A, see, *e.g.*, Claims 1 and 45, and the claims depending from these independent claims. In this method, a test compound is incubated with a cell containing a BoNT/A light chain localized to the cytoplasmic side of the plasma membrane and changes in the intracellular localization pattern of the toxin over time are determined, see Claims 1 and 45. Cells contacted with the test compound that show a reduction of membrane-bound BoNT/A light over time relative to a control cell indicates a compound that reduces biological persistence. Cells contacted with the test compound that show an increase of membrane-bound BoNT/A light over time relative to a control cell indicates a compound that enhances of biological persistence. As such, the presently claimed methods require a cell to be engineered to possess BoNT/A light chains that are already associated with the plasma membrane. The present specification indicates that this can be achieved by either transiently or stably expressing the BoNT/A light chain in the cell from an expression construct or directly transfecting the light chain into the cell, see, *e.g.*, ¶¶ 63-67. Because the cell is engineered to have the BoNT/A light chain present on the cytoplasmic side of the plasma membrane, the cells are in a continuous state of intoxication because the light chain can freely cleave its SNARE target protein.

Claim 60 of the Li publication recites, in part, "[a] method of identifying a compound that alters internalization of a Clostridial toxin into a cell." In this method, a Clostridial toxin in conjunction with a test compound is applied to a cell and changes in the internalization of the toxin relative to a toxin without the test compound is determined, see Claim 60. Cells that exhibit a decrease in intoxication characteristics indicate that the test compound reduces the toxin's ability to internalize into the cell. Conversely, cells that exhibit an increase in intoxication characteristics indicate that the test compound enhances the toxin's ability to internalize into the cell. Thus, the assay of Claim 60 relies on the ability to distinguish between cells that exhibit intoxication characteristics as manifested by the presence of the toxin's light chain within the cell from those cells that do not exhibit intoxication characteristics as manifested by the absence of the toxin's light chain within the cell.

The Herreros reference discloses that the H_C cell binding domain of TeNT interacts with Thy-1, a protein found in lift rafts, see, *e.g.*, abstract. At best, this reference identifies one of

the protein components associated with a receptor complex located in a lipid raft that can bind to TeNT. Thus, this reference adds nothing to either of the methods discussed above. As such, the Herreros reference does not contradict the teaching of the Li publication.

Modifying the cell used in the assay claimed in the Li publication so that it comprises an internally localized BoNT/A light chain as presently claimed would result in a cell that continuously displays intoxication characteristics. As such the BoNT/A light chain can freely cleave its SNARE target because it has bypassed the internalization process and, in a sense, has already been "internalized." Thus, the ability of a test compound to alter the internalization of a Clostridial toxin into a cell cannot be measured since the cell is in a state that indicates it is maximally intoxicated. As such, including a BoNT/A light chain in a cell would make the internalization assay disclosed in the Li publication inoperable for its indented use because it could not distinguish test compound that affect the internalization of a Clostridial toxin. Therefore, the Applicants respectfully submit that a *prima facie* case of obviousness cannot be made because combining the Li publication with the Herreros reference as suggested by the Examiner would result in an inoperable internalization assay.

Li and Herrero combination changes the principle of operation of Li.

According to *MPEP* §2143.01, if a proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious. The Applicants respectfully submit that it would not be obvious for one of ordinary skill in the art to combine the cited references because combining the Li publication with the Herrero reference would change the principle of operation of the internalization assay disclosed in the Li publication.

A Clostridial toxin is an approximately 150 kDa protein comprising a heavy chain and a light chain. The heavy change contains two major functional domains. The carboxyl-terminal half includes a receptor binding domain (H_C cell binding domain), whereas, the amino-terminal half contains a translocation domain (H_N translocation domain), see present application at ¶¶ 13-14. The light chain includes a zinc-dependant endoprotease domain

(enzymatic domain), see present application at ¶ 15. The intoxication of a cell by a Clostridial toxin is a multi-step process involving: 1) binding of the Clostridial toxin via the H_C cell binding domain to a cell surface receptor; 2) internalization of the toxin-receptor complex into a cell, thereby forming an endosome; 3) disassociation of the toxin light chain from the heavy chain; 4) release of the toxin light chain from the endosome into the cytoplasm through a pore formed by the H_N translocation domain; 5) subcellular localization of the light chain to its target SNARE protein; and 6) proteolytic cleavage of the target SNARE by the enzymatic domain, see present application at ¶¶ 13-16.

The presently claimed methods are directed toward a cell-based light-chain localization assay useful for identifying a compound that either reduces or increases a biological persistence of a BoNT/A, see, *e.g.*, Claims 1 and 45. The basis for this assay is whether or not a test compound can alter the length in time a BoNT/A light chain remains associated with the cytoplasmic side of the plasma membrane. Cells contacted with the test compound that show a reduction of membrane-bound BoNT/A light over time relative to a control cell indicates a compound that reduces biological persistence. Cells contacted with the test compound that show an increase of membrane-bound BoNT/A light over time relative to a control cell indicates a compound that enhances of biological persistence, see, *e.g.*, Claims 1 and 45.

Claim 60 of the Li publication recites, in part, "[a] method of identifying a compound that alters internalization of a Clostridial toxin into a cell." This assay relies on steps 1 and 2 of the intoxication process and measures whether or not a test compound can alter the ability of a Clostridial toxin to bind to its cognate receptor and/or alter the internalization of the toxin-receptor complex into a cell, events that occur on the extracellular side of the plasma membrane, see, *e.g.*, the Li publication at ¶¶ 36, 46, 62, and 120. The basis for the altered internalization of the toxin relies on whether the test compound alters the formation of the lipid rafts that participate in the internalization of the toxin. Test compounds that disrupt lipid raft formation will reduce the ability of a Clostridial toxin to bind and/or internalize into a cell. Test compounds that promote lipid raft formation will increase the ability of a Clostridial toxin to bind and/or internalize into a cell.

As discussed above, the Herreros reference adds nothing to either of the methods discussed above and thus does not contradict the teaching of the Li publication.

The suggested combining of the Li publication with the Herreros reference would change the principle of operation of the assay disclosed in the Li publication. The Li publication relies on the internalization mechanism of the 150 kDa toxin that binds and internalizes a toxin into a cell and whether or not a test compound can alter this mechanism. As such, this assay measures the capacity of a test compound to alter the internalization of a Clostridial toxin during the intoxication process, specifically steps 1 and 2 described above. However, the ability of a test compound to reduce or enhance internalization of a toxin has no bearing on the biological persistence of that internalized toxin. For example, lets say that administration of 50 units of a BoNT/A to treat a muscle spasm disorder has an average effective duration of about 3 months. A test compound identified using the Li publication assay that promotes lipid raft formation means that a lower dose of a BoNT/A (25 units) in conjunction with the test compound can be used to treat the muscle spasm disorder just as effectively as the original higher dose (50 units) because the test compound enhances BoNT/A internalization. However, the biological persistence of the internalized toxin will still be only 3 months in duration.

On the other hand, the presently claimed methods relies on the membrane-association ability of a BoNT/A light chain that enables a light chain to remain associated with the cytoplasmic side of the plasma membrane after intoxication and whether or not a test compound can alter this association. As such, this assay measures the capacity of a test compound to alter events subsequent to the intoxication process, *i.e.*, the presently claimed method has nothing at all to do with the internalization process as described above. The present method is useful for determining how many days a cell remains intoxicated after the intoxication event and test compounds that alter the duration of this biological persistence. Using the muscle spasm disorder example above, a test compound identified using the presently claimed method that enhances biological persistence means that the same dose of a BoNT/A (50 units) can be used to treat the muscle spasm disorder for a longer period of time, say 4 months, because the biological persistence of the internalized toxin is enhanced.

Thus, the Applicants respectfully disagree with the Examiner's contention that "affecting internalization of a toxin by altering affinity to lipid rafts for toxin internalization would affect biological persistence of the toxin," see May 26, 2006 Office Action at pg. 16, lines 19-21. In addition, the Applicant's respectfully disagree with the Examiner's statement that "the biological persistence of a toxin is mainly dependant on the binding of the heavy chain to lipid rafts for internalization and the enzymatic activity of the light chain," see May 26, 2006 Office Action at pg. 16, line 21 through pg. 17, line 1. The ability of a Clostridial toxin to intoxicate a cell is not material to the presently claimed invention. In fact, the heavy chain of a toxin is not required to practice the claimed invention at all. As disclosed at ¶¶ 64-67, the cell comprises a light chain can be accomplished by an expression construct encoding just the light chain.

Therefore, the Applicants respectfully submit that a *prima facie* case of obviousness-type double patenting cannot be made because the combining the Li publication with the Herreros reference as suggested by the Examiner would change the principle of operation of the internalization assay disclosed in the Li publication.

Conclusion

For the reasons stated above, the Applicants respectfully submit that the assertion of obviousness-type double patenting is unsupported by the cited references because the suggested modifications and combinations required to produce the presently claimed method 1) would result in an inoperable internalization assay as disclosed in the Li publication; and 2) would change the principle of operation of the internalization assay as claimed in the Li publication. Therefore, the Applicants respectfully request withdrawal of the 35 U.S.C. § 101 obviousness-type double patenting rejection for Claims 1, 3-20, 22, 45-47, 56 and 57.

Rejection Pursuant to 35 U.S.C. § 103(a) Obviousness

I. Obviousness rejections over Schmidt in view of Fernandez-Salas I and Fernandez-Salas II

The Examiner has rejected Claims 1, 3-20, 22, 45-47, 56 and 57 as allegedly being obvious under 35 U.S.C. § 103(a) over James J. Schmidt and Robert G. Stafford, *High Throughput Assays for the Proteolytic Activities of Clostridium Neurotoxins*, U.S. Patent 6,762,280 (effective filing date Sep. 25, 2000), hereafter the "Schmidt patent" in view of Ester Fernandez-Salas et al., *Plasma Membrane Localization Signals in the Light Chain of Botulinum Neurotoxin Serotype A*, ABS 9.2 Soc. Neurosci. Abstr. Viewer Itiner. (Nov., 2003), hereafter the "Fernandez-Salas I abstract"; or Ester Fernandez-Salas et al., *Localization of BoNT Light Chains in Neuronal and Non-Neuronal Cell Lines, Implications for the Duration of Action of the Different Serotypes*, 365(Suppl. 2) Naunyn-Schmiedeberg Arch. Pharmacol. ABS R19 (Jun., 2002), hereafter the "Fernandez-Salas II abstract".

The Examiner contends that it would have been *prima facie* obvious to a person of ordinary skill in the art at the time the invention was made to combine the teaching of these references and come up with a method of identifying a compound that alters a biological persistence of a BoNT/A as presently claimed. Specifically, the Examiner argues that it would have been obvious to modify the method for identifying a compound that inhibits or enhances the proteolytic activity of BoNT/A disclosed in the Schmidt patent to further measure biological persistence of a BoNT/A because the Fernandez-Salas I abstract and Fernandez-Salas II abstract discloses that BoNT/A light chain co-localizes with SNAP-25. The Applicants respectfully ask for reconsideration under 37 C.F.R. § 1.111.

Reference Citation

As a general note, the Examiner has cited Steward et al., Naunyn-Schmiedeberg's Archives of Pharmacology (June 2002) Vol. 365 No. Supplement 2, pp. R19. However, the authorship of the reference cited indicates that Fernandez-Salas as first author, see citation above. The Applicants have included a copy of this citation and respectfully request that the

Examiner confirm that this Fernandez-Salas abstract is the reference being cited against the present application.

Schmidt, Fernandez-Salas I and Fernandez-Salas II combination makes Schmidt unsatisfactory for its intended use.

According to MPEP §2143.01, if proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification. The Applicants respectfully submit that it would not be obvious for one of ordinary skill in the art to combine the cited references because combining the Schmidt patent with the Fernandez-Salas I abstract and Fernandez-Salas II abstract would render the substrate-release assay disclosed in the Schmidt patent as inoperable for its intended use because such a combination would prevent the ability of the assay to determine proteolytic activity.

The presently claimed methods are directed toward a cell-based light-chain localization assay useful for identifying a compound that either reduces or increases a biological persistence of a BoNT/A, see, *e.g.*, Claims 1 and 45, and the claims depending from these independent claims. In this method, a test compound is incubated with a cell containing a BoNT/A light chain localized to the cytoplasmic side of the plasma membrane and changes in the intracellular localization pattern of the toxin over time are determined, see Claims 1 and 45. The BoNT/A light chain can be expressed by the cell from an expression construct or transfected into the cell, see, *e.g.*, ¶¶ 63-67. Cells contacted with the test compound that show a reduction of membrane-bound BoNT/A light over time relative to a control cell indicate a compound that reduces biological persistence. Cells contacted with the test compound that show an increase of membrane-bound BoNT/A light over time relative to a control cell indicate a compound that enhances of biological persistence.

The Schmidt patent discloses an in vitro substrate-release assay useful for identifying a compound that inhibits or enhances the proteolytic activity of BoNT/A, see, col. 9, line 65 through col. 10, line12; and Claim 9. In this method, a botulinum toxin is “incubated with a test compound . . . transferred to solid supports onto which is immobilized a peptide

substrate for the BoNT enzyme being tested . . . and processed," see col. 10, lines 1-5. The immobilized peptide substrate is a SNARE peptide that contains a reactive group at its C-terminal end used to attach the substrate to the solid support, a fluorophore group at its N-terminal end used in the fluorescence detection method, and an intervening BoNT cleavage site, col. 7, lines 15-20; and col. 7, lines 26-56. The Schmidt patent indicates that "a reduction in the ability of the toxin to cleave the peptide substrate relative to unincubated toxin indicates a[n] inhibitory compound," see, col. 10, lines 6-9. On the other hand, "an increase in the ability of the toxin to cleave the peptide substrate relative to unincubated toxin indicated a stimulatory compound," see col. 10, lines 9-12.

The Fernandez-Salas I abstract discloses that BoNT/A light chains localize in the plasma membrane of neurons in the same compartment as SNAP-25 and that signals present on the N-terminus and C-terminus of the BoNT/A light chain mediate this localization.

The Fernandez-Salas II abstract discloses that BoNT/A light chains localize in the plasma membrane of neuronal and non-neuronal cells in the same compartment as SNAP-25.

Replacing the immobilized SNARE substrate with a BoNT/A light chain on the solid support, as suggested by the Examiner, would result in an inoperable substrate-release assay as disclosed in the Schmidt patent. The Schmidt patent method relies on the ability of the BoNT to cleave the SNARE substrate immobilized on the solid support. If there is no SNARE protein, there is no substrate to be cleaved by the toxin, and thus no way of determining to what extent a test compound decreases or increases the amount of substrate proteolytically cleaved by a botulinum toxin light chain. Additionally, adding a BoNT/A light chain to the solid support, so that both the SNARE substrate and the BoNT/A light chain are present on the solid support, is also inoperable. The presence of the BoNT/A light chain on the support would result in the SNARE substrate being continuously cleaved by the toxin. As such, the influence that a test compound may have on a Clostridial toxin's proteolytic activity will be completely masked because the assay will always indicate that the applied toxin has full proteolytic activity.

Thus, replacement of a SNARE substrate with a BoNT/A light chain would make the substrate-release assay disclosed in the Schmidt patent inoperable for its indented use. Therefore, the Applicants respectfully submit that a *prima facie* case of obviousness cannot be made because combining the Schmidt patent with the Fernandez-Salas I abstract and Fernandez-Salas II abstract as suggested by the Examiner would result in an inoperable substrate-release assay.

Schmidt, Fernandez-Salas I and Fernandez-Salas II combination changes the principle of operation of Schmidt.

According to MPEP §2143.01, if a proposed modification or combination of the prior art would change the principle of operation of the prior art invention being modified, then the teachings of the references are not sufficient to render the claims *prima facie* obvious. The Applicants respectfully submit that it would not be obvious for one of ordinary skill in the art to combine the cited references because combining the Schmidt patent with the Fernandez-Salas I abstract and Fernandez-Salas II abstract would change the principle of operation of the substrate-release assay disclosed in the Schmidt patent.

As discussed above, the presently claimed method comprises a cell-based light-chain localization assay useful for identifying a compound that reduces or enhances a biological persistence of a BoNT/A.

As discussed above, the Schmidt patent discloses an in vitro substrate-release method useful for identifying a compound that inhibits or enhances the proteolytic activity of BoNT/A. In this method, a solid support is made that contains a SNARE protein that serves as the substrate for a BoNT. A BoNT is incubated with a test compound in solution and this solution is then applied to the solid support containing the SNARE substrate. As the BoNT comes in contact with its immobilized SNARE substrate, the toxin cleaves it, whereby the cleaved SNARE product is released from the solid support and collected. The ability of a test compound to alter the amount of proteolytic cleavage is the basis for determining whether a test compound inhibits BoNT activity (*i.e.*, reduced proteolytic cleavage relative to

a control) or enhances proteolytic cleavage (*i.e.*, increases proteolytic cleavage relative to a control).

As discussed above, both the Fernandez-Salas I abstract and the Fernandez-Salas II abstract discloses that BoNT/A light chains localize in the plasma membrane of neuronal and non-neuronal cells in the same compartment as SNAP-25.

The suggested combining of the Schmidt patent with the Fernandez-Salas I abstract and Fernandez-Salas II abstract would change the principle of operation of the assays disclosed in the Schmidt patent in at least two ways.

First, the molecule used as the read-out of a test compound's activity between the Schmidt patent and the present claimed methods operates under a different principle. The Schmidt patent discloses a substrate-release assay that uses a SNARE protein substrate which serves as a read-out for the proteolytic activity of the BoNT, referred to as a Type (II) Substrate, see, col. 7, line 14 through col. 9, line 34. As such, the principle of operation is to determine to what extent a test compound can decrease or increase the amount of SNARE substrate proteolytically cleaved by a botulinum toxin light chain. On the other hand, the presently claimed methods are directed toward a toxin localization assay that uses the length of time that a BoNT/A light chain remains associated with the plasma membrane as a read-out for biological persistence of the BoNT. As such, the principle of operation of the read-out is to determine to what extent a test compound decreases or increases the length of time a BoNT/A light chain remains associated with the plasma membrane. Thus, the principle of operation between these two methods are different because the Schmidt patent assay relies on the level of enzymatic proteolysis of a substrate, whereas, the presently claimed method relies on the length of time that a toxin remains associated with a membrane.

Second, the mechanics of the assay between the Schmidt patent and the present claimed methods operates under a different principle. The Schmidt patent discloses an in vitro substrate release assay where the SNARE substrate is immobilized on a solid support, a solution containing a BoNT and a test compound is poured over this solid support, the flow-

through is collected and then tested for fluorescence, see, col. 4, lines 57-67; and col. 8, line 57 through col. 9, line 16. The SNARE substrates contain a reactive group at its C-terminal end used to attach the substrate to the solid support, a fluorophore group at its N-terminal end used in the fluorescence detection method, and an intervening BoNT cleavage site, col. 7, lines 15-20; and col. 7, lines 26-56. For this assay to work, the cleavage product must be removed from the solid support matrix and transferred to a separate container, see, col. 4, lines 23-30. This is because both cleaved products and uncleaved SNARE substrates contain the fluorophore and if they remain together in would be impossible to distinguished the substrates from the products using a fluorimeter. As such, the assay cannot be performed in a cell because there would be no way to separate the cleaved product from the uncleaved SNARE substrate. Thus, the principle of operation of the assay relies on an in vitro-based, solid support matrix that enables the separation of the cleaved product from the uncleaved SNARE substrate. On the other hand, the presently claimed method is a cell-based toxin localization assay where a cell containing a BoNT/A light chain is exposed to a test compound and the localization pattern of the BoNT/A light chain is determined over time. This method does not require the separation of the BoNT/A light chain from the cell. Additionally, this method requires a cell and cannot be performed as an in vitro assay because of the essential requirement that a BoNT/A light chain be associated with the plasma membrane. Thus, the principle of operation between these two methods are different because the Schmidt patent assay relies on the in vitro assay that requires the separation of cleavage products from the uncleaved substrates, whereas, the presently claimed method relies on a cell-based assay that requires a differential localization of a toxin over time.

Thus, replacing the SNARE substrate with the BoNT/A light chain would change the principle of operation from an in vitro enzymatic-based assay that uses changes in substrate cleavage amounts as the principle of operation to a cell-based toxin localization assay that uses changes in the membrane localization pattern of a toxin as the principle of operation. In order for the substrate-release assay disclosed in the Schmidt patent to incorporate the suggested modifications, the substrate-release assay would have to change 1) its read-out from one that relies on the level of enzymatic proteolysis of a substrate to one that relies on the length of time that a toxin remains associated with a membrane; 2) its mechanics from

one that relies on an in vitro based assay that requires separation of a product from its substrate to one that relies on a cell-based assay that requires differential patterns of membrane localization of a toxin over time.

Therefore, the Applicants respectfully submit that a *prima facie* case of obviousness cannot be made because the combining the Schmidt patent with the Fernandez-Salas I abstract and Fernandez-Salas II abstract as suggested by the Examiner would change the principle of operation of the substrate-release assay disclosed in the Schmidt patent.

Schmidt, Fernandez-Salas I and Fernandez-Salas II provide no teaching, suggestion or motivation to combined references.

According to MPEP § 2143.01, obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either explicitly or implicitly in the references themselves or in the knowledge generally available to one of ordinary skill in the art. The Applicants respectfully submit that a *prima facie* obviousness case fails because the Schmidt patent, the Fernandez-Salas I abstract and Fernandez-Salas II abstract do not provide any motivation, suggestion or teaching that would lead a person skilled in the art to specifically make a method of identifying a compound that either reduces or enhances the biological persistence of BoNT/A as presently claimed.

As discussed above, the presently claimed method comprises a cell-based light-chain localization assay useful for identifying a compound that reduces or enhances a biological persistence of a BoNT/A. The present specification defines biological persistence as "the continuous period of time that a light chain retains its enzymatic activity when that light chain is within a cell or outside of a cell," see pg. 11, ¶ 34. As such, a test compound identified from presently claimed method must exhibit an activity that either shortens the time period that a BoNT/A is active or lengthens the time period that a BoNT/A is active.

The test localization assay does not measure the degree of BoNT/A proteolytic activity that a compound alters at any given time, as does, *e.g.*, the test enzymatic assay recited in

pending Claim 16, a dependent claim that further adds the steps of determining BoNT/A proteolytic activity of the test localization assay recited in independent Claim 1. See also the present specification at pg. 20, 70 through pg. 22, 77. Proteolytic activity measures whether a toxin can cleave a substrate at all and is a completely independent measurement of how long a toxin can persist over time. Thus, a test compound that alters the biological persistence of a BoNT/A light chain and a test compound that alters the proteolytic activity of a BoNT/A light chain are two separate properties. For example, a test compound may significantly alter the biological persistence of a BoNT/A light chain and yet have no affect on the proteolytic activity of the toxin. Likewise, a test compound may significantly alter the proteolytic activity of a BoNT/A light chain and yet have no affect on the biological persistence of the toxin.

As discussed above, the Schmidt patent discloses an in vitro substrate-release assay useful for identifying a test compound that inhibits or enhances the proteolytic activity of BoNT/A. As such, the Schmidt patent is completely silent with respect to any disclosure for a cell-based light-chain localization assay, let alone such an assay useful for identifying a compound that reduces or enhances a biological persistence of a BoNT/A. Thus, to sustain a *prima facie* case of obviousness the Fernandez-Salas I abstract and the Fernandez-Salas II abstract, at a minimum, need to teach, suggest or motivate a person of ordinary skill in the art to develop 1) a cell-based assay useful for identifying a compound that reduces or enhances a biological persistence of a BoNT/A; 2) a cell-based assay that relies on the membrane localization pattern of a BoNT/A light chain as a read-out; and 3) a cell-based BoNT/A membrane localization assay useful for identifying a compound that reduces or enhances a biological persistence of a BoNT/A.

The Fernandez-Salas I abstract discloses that BoNT/A light chains localize in the plasma membrane of neurons in the same compartment as SNAP-25. The Fernandez-Salas I abstract is directed at solving the problem of identifying what molecular mechanisms contained within BoNT/A controls its biological persistence and not how to identify a compound that alters the biological persistence of a BoNT/A. As such, the Fernandez-Salas I abstract provides absolutely no information regarding any assay useful for identifying any

compound that alters any activity, let alone a cell-based assay useful for identifying a test compound that either reduces or enhances biological persistence of a BoNT/A.

Thus, the Fernandez-Salas I abstract does not teach, suggest or motivate a person of ordinary skill in the art to develop 1) a cell-based assay useful for identifying a compound that reduces or enhances a biological persistence of a BoNT/A; 2) a cell-based assay that relies on the membrane localization pattern of a BoNT/A light chain as a read-out; and 3) a cell-based BoNT/A membrane localization assay useful for identifying a compound that reduces or enhances a biological persistence of a BoNT/A. Thus, the Fernandez-Salas I abstract does not overcome any of the three deficiencies of the Schmidt patent and a *prima facie* case of obviousness cannot be sustained.

The Fernandez-Salas II abstract discloses that BoNT/A light chains localize in the plasma membrane of neuronal and non-neuronal cells in the same compartment as SNAP-25. As such, this abstract suffers to an even greater extent than the Fernandez-Salas I abstract. Thus, the Fernandez-Salas II abstract fails to teach, suggest or motivate a person skilled in the art to develop 1) a cell-based assay useful for identifying a compound that reduces or enhances a biological persistence of a BoNT/A; 2) a cell-based assay that relies on the membrane localization pattern of a BoNT/A light chain as a read-out; and 3) a cell-based BoNT/A membrane localization assay useful for identifying a compound that reduces or enhances a biological persistence of a BoNT/A. Thus, the Fernandez-Salas II abstract, either alone or with the Fernandez-Salas I abstract, does not overcome any of the three deficiencies of the Schmidt patent and a *prima facie* case of obviousness cannot be sustained.

Thus, the Applicants respectfully submit that the assertion of obviousness is unsupported by the cited references because none of these references provide any teaching, suggestion or motivation to develop 1) a cell-based assay useful for identifying a compound that reduces or enhances a biological persistence of a BoNT/A; 2) a cell-based assay that relies on the membrane localization pattern of a BoNT/A light chain as a read-out; and 3) a cell-based BoNT/A membrane localization assay useful for identifying a compound that reduces or enhances a biological persistence of a BoNT/A, in order to arrive at the presently claimed

invention. Therefore, the Applicants respectfully submit that a *prima facie* case of obviousness cannot be made because the combining the Schmidt patent with the Fernandez-Salas I abstract and Fernandez-Salas II abstract as suggested by the Examiner because these references do not provide any motivation, suggestion or teaching that would lead a person of ordinary skill in the art to specifically make the presently claimed methods.

Conclusion

For the reasons stated above, the Applicants respectfully submit that the assertion of obviousness is unsupported by the cited references because the suggested modifications and combinations required to produce the presently claimed method 1) would result in an inoperable substrate-release assay as disclosed in the Schmidt patent; 2) would change the principle of operation of the substrate-release assay as taught in the Schmidt patent; and 3) provide no motivation, suggestion or teaching that would lead a person of ordinary skill in the art to specifically make the presently claimed methods. Therefore, the Applicants respectfully request withdrawal of the 35 U.S.C. § 103(a) obviousness rejection for Claims 1, 3-20, 22, 45-47, 56 and 57.

CONCLUSION

For the above reasons the Applicants respectfully submit that the claims are in condition for allowance, and the Applicants respectfully urge the Examiner to issue a Notice to that effect. Should there be any questions, the Examiner is invited to call the undersigned agent.

Please use Deposit Account 01-0885 for the payment of any extension of time fees under 37 C.F.R. § 1.136 or any other fees due in connection with the current response.

Respectfully submitted,

/Dean G. Stathakis/

Dean G. Stathakis, Ph.D.
Registration No. 54,465
Agent of Record

ALLERGAN
LEGAL DEPARTMENT
2525 Dupont Drive
Irvine, California 92612-1599
Tel: 714/246-6521
Fax: 714/246-4249